ANDROGEN DEPENDENCY OF THE ANDROGEN RECEPTOR IN RAT EPIDIDYMIS

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SUMMARY.Binding of radioactive androgen to the 8S epididymal receptor is reduced to undetectable levels after 30 days of castration.Testosterone propionate(200 µg/day)administered starting immediately after castration and continued for 30 days prevented the disappearance of binding activity.Furthermore,testosterone propionate given for 11 days begining on day 30 after castration was able to reinduce binding of radioactive androgen to the 8S receptor.We conclude that the binding of androgen to the epididymal citoplasmatic receptor is under androgenic control.

The structural and functional integrity of the epididymis are dependent on the adequate provision of androgens(1,2).Available experimental evidence indicates that the initial step of the complex mechanism by which the hormones exert their action on this target tissue involves interaction with specific citoplasmatic(3,4)and nuclear receptors(5,6).In a recent report(7)we have shown that castration in the adult rat led to a gradual decrease in the number of cytoplasmatic binding sites for androgens,reaching undetectable levels after 25 days of castration had elapsed.However,after 30 days of unilateral castration binding to the receptor on the castrated side was preserved(7),suggesting an effect of circulating hormones secreted by the controlateral testis.These suggestions prompted the investigation reported on this paper on the effect of androgens on their own epididymal receptor.

METHODS AND MATERIALS

Male wistar rats weighing 250-300 g were routinely anesthetized with ether and castrated through a scrotal incision.

a)In vitro studies:a group of 16 rats in 4 separate experiments was studied 30 days after castration;a second group of 10 rats in 5 experiments was cas-

trated and given 0.2 mg/day of testosterone propionate(TP) for 11 days starting on the 31st.day after castration and sacrificed 48 h after the last injection.Epididymides were homogenized in 2 volumes(w:v) Of 20 mM Tris-HCl buffer,pH 7.4 containing 1.5 mM EDTA and 0.25 mM DTT,and the homogenate centrifuged at 105,000 x g for 1 h.

Aliquots of cytosol containing 2 mg protein were diluted to a final volume of 400 µl and incubated with [1,2-^{3}H]- dihydrotestosterone(0.067 µCi,4 mM) for 2 hours at 0-°C.These were layered over 5-20 % linear sucrose gradients and spun at 115,000 x g for 15 h at 0-°C in a SW 56 rotor(Spinco L2-65B).Gradients were fractioned into 30 samples and radioactivity determined in Packard Scintillation Counter.

b)In vivo studies:a first group of 8 rats in 4 separate experiments were castrated for 30 days;a second group of 6 animals was castrated and given 0.2 mg/day of TP for 30 days starting on the first day after orchidectomy; a third group of 6 animals was castrated and given the same dose of TP for 11 days starting on the 31st day after orchidectomy.The second and third groups were sacrificed 48 h .after the last injection and the animals studied individually.Two hours prior to sacrifice rats were eviscerated,functionally hepatectomized and injected iv with 50 µCi of [1,2,6,7,-^{3}H]-testosterone. Preparation of cytosol,gradient ultracentrifugation and fractionation were carried out as described for the in vitro experiments.

RESULTS

To verify the finding that castration for a period of 30 days resulted in undetectable levels of binding of radioactive androgen to the 8S cytoplasmatic receptor of rat epididymis(7),we attempted to label the receptors injecting the hormone "in vivo",in the belief that this would result in a better preservation of the receptor during the isolation procedures. Androgen replacement(TP) was administered to castrated rats following two schedules,a)"maintenance":when the androgen treatment was begun immediately after castration and continued for 30 days , and b)"reinduction":when treatment was begun after 30 days of castration and continued for 11 days.

Figure 1 illustrates the results of the in vivo experiments.The profile of the sucrose gradient centrifugation of cytosol shows the absence of bind-

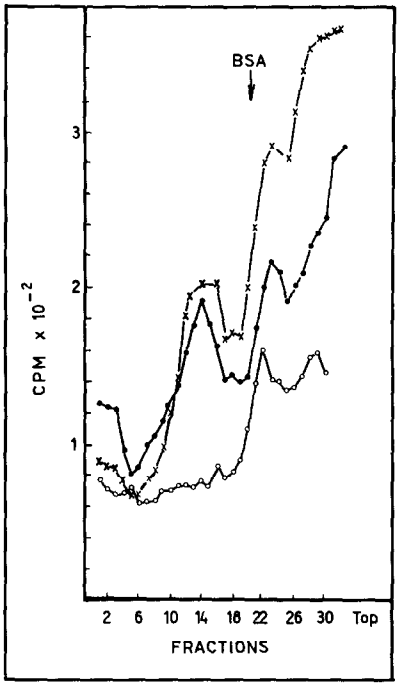

FIGURE 1: Sucrose gradient ultracentrifugation analysis of epididymal cytosol fractions obtained after "in vivo" labelling. O—O castrated for 30 days, ●—● castrated rats maintained with testosterone propionate for 30 days, ✕—✕ castrated for 30 days followed by administration of testosterone propionate for 11 days. Rats were eviscerated, functionally hepatectomized, injected iv with [^{3}H]- testosterone and sacrificed 2 h. later. Epididymal cytosol was prepared as described in the text.

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ng to 8S macromolecules after 30 days of castration, in contrast to what is usually seen in intact rats(3). However, TP treatment administered following the "maintenance" schedule prevented the decline of receptor binding sites and, furthermore, TP was able to reinduce binding sites which had disappeared after castration.

These experiments were also performed with "in vitro" labelling of the cytosol and the results are shown in Figure 2. Again, there is no detectable binding to the 8S receptor in the sample obtained from rats castrated for 30 days. In contrast, the cytosol from rats which received TP according to the "reinduction" schedule presents a conspicuous peak of radioactivity sedimenting bound to macromolecules in the 8S zone.

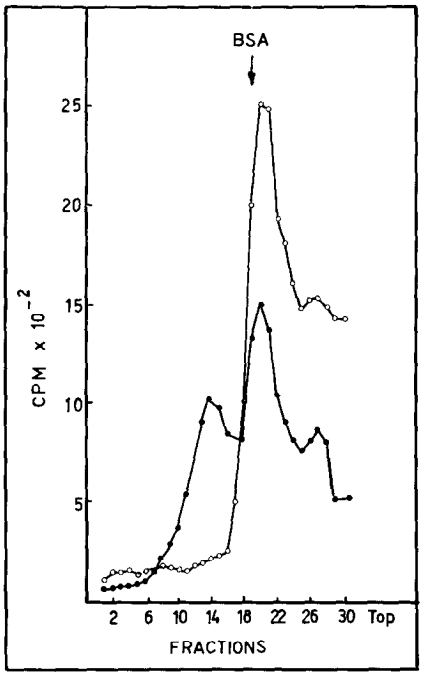

FIGURE 2: Sucrose gradient ultracentrifugation analysis of epididymal cytosol fractions incubated "in vitro" with ^{3}H-dihydrotestosterone. O-O castrated for 30 days, ●-● castrated for 30 days followed by administration of testosterone propionate for 11 days. BSA=bovine serum albumin.

DISCUSSION

The results reported in this paper demonstrate that androgens can prevent the decrease of the epididymal 8S cytoplasmatic receptor brought about by castration and also reinduce the receptor which had declined in the castrated animal.

The obvious conclusion to be drawn from these results is that binding of androgens to the epididymal receptor is androgen-dependent. It would be tempting to speculate that the receptor is an inducible protein under the inductive control of its own ligand. However, such a theory could not be proposed until knowledge on whether androgens stimulate the synthesis of receptor or activate a preformed precursor is gathered. Nevertheless, supporting this contention, our previous results (9) demonstrate a close correlation between the occurence of detectable levels of endogenous dihydrotestosterone and the development of 8S receptor binding activity during sexual maturation in the

rat.On the other hand,we only know for certain that the regression of the epididymal weight and total protein content induced by castration are partially prevented by androgen administration(7).

Tindall et al (8) did not find any indication of selective dependence of the epididymal cytosol receptor on androgens.In their hands,binding to receptors was not affected by 15 days of castration or 8 days of androgen treatment to the castrate.However,we have reported(7) that at least 25 days of castration were necessary for the receptor to become undetectable in the epididymis

Examples of receptor induction by steroid hormones can be found in the work of Milgrom et al(10) and Leavitt et al(11) who have shown the uterine progesterone receptors to be induced by estrogen and repressed by progesterone itself.Also,Roy et al(12)have described the induction by androgen hepatic receptor for androgen in the rat.

If androgen action is dependent on binding to intracellular receptors it seems reasonable to presume that they should be present prior to the androgenic stimulus.Therefore,although specific receptors are genetically determined components of the target cell,their concentration(binding sites/cell)might be regulated by the hormone.

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